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Spray Method, A New Technique for Investigation of Vessels on the Tissue Surface.

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"Spray method", a new technique is explained. This method is useful for observation of vessels and fibers on tissue surfaces, especially of brain. It is particularly useful in situations where intravascular injection is not possible, and can be combined easily with other methods without hindering their effectiveness.

Many useful methods for the investigation of the vessels on the surface of tissue are known, but most of them require fresh material. In usual practice, however, material becomes available for study only after some time has elapsed and frequently the vessels contain coagulated material. This type of specimen, then, may not lend itself to the usual method of the intravascular injection of colored material. Thus one might say that there are few good methods applicable for every case.

Recently in the course of investigation of the vasculature of the human lateral geniculate body the author often found that intravascular injection was very difficult. Whereas the injected material would fill most of the small sized vessels, frequently it would not enter the capillaries and precapillaries. The material studied had been fixed with formalin introduced by the systemic irrigation method, but some of the coagulated vascular contents remained in this part of the brain. On one occasion the author tried the injection of methylene blue solution into a small vessel with the specimen immersed in water. This was carried out under a dissecting microscope. During the injection some of the dye leaked from the vessel and impregnated the vessel wall, even into the fine branches. Thus the outline of the vascular tree was beautifully seen. The method was subsequently refined and it is the purpose of this paper to present the technique and some of its results.

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METHOD

1. Equipment

1) A deep Petri dish to serve as water bath.
2) Dilute methylene blue solution 1 : 5000.
3) One 5 ml. and one 20 ml. syringe.

As can be seen, the equipment required is simple, and colored solution other than methylene blue could be used. It may be necessary to use a magnifying glass or dissecting microscope.

2. Method

The piece of tissue to be studied is put into the water bath. While observing through the dissecting microscope a stream of dye solution is sprayed from the 5 ml. syringe onto the surface of the tissue which is kept immersed in water. The excess dye is then washed away with plain water by means of the 20 ml. syringe. By doing this the cloud of dye in the water is dispersed and the tissue can be viewed through the dissecting microscope as one sees the bed of the ocean through under water goggles.

RESULTS AND COMMENT

The following series of photographs will illustrate the effectiveness of the method.

Case 1., Fig. 1.: A view of the human pulvinar surface magnified 26 times. Before staining, the surface structure and vessels are not visible and the surface is white and appears homogenous.

Fig. 2.: After spraying a small amount of methylene blue solution, some medium sized vessels are visible.

Fig. 3.: The more methylene blue solution has been sprayed on, the more vessels are visible.

Fig. 4.: After spraying 10 ml. of dilute methylene blue solution. Fine capillaries are also visible.

Case 2., Fig. 5.: A view of the medial surface of the human occipital lobe magnified 27 times. Before staining, some of the contents of the small vessels have coagulated. These are visible as black lines.

Fig. 6.: The syringe is on the right side of the picture (top of the needle is visible) and dye is sprayed toward the right half.

Fig. 7.: The right half has taken the stain and small vessels become visible. Compare this with the left half.

Fig. 8.: Higher magnification of figure 7. Small vessels are seen.

Case 3., Fig. 9.: A view of the anterior part of the human lateral geniculate body magnified 10 times. The vessels and the sites of

their entrance into the brain tissue are demonstrated by a combination of intravascular injection and the spray method.

Fig. 10.: After three minutes have elapsed the dye has faded.

If one wishes to decolorize the stained tissue this can be achieved either by allowing it to stand for several hours in water or by dropping drops of absolute alcohol and water alternately on the surface (decolorization).

Case 4., Fig. 11.: A view of the stained surface of the lateral geniculate body magnified 9 times.

Case 5., Fig. 12.: A view of the stained vessels in the pulvinar magnified 22 times.

Applications of the method:

The structures on the surface of the tissue can be visualized. Such structures include vessels on the surface, fibers, small projecting masses, and any clumps of precipitated material.

In addition the pattern of the surface structures can be visualized. The method, of course, is not applicable to specimens whose surface is covered with exudate or gelatinous material.

Advantages of the method:

1) Vessels can be visualized even if the lumen is occluded by thrombi or other obstructing materials.

2) Any portion may be studied, even the very small fragments of tissue (In the ordinary intravascular injection method it is necessary to have a main vessel with a considerable caliber and a specimen must be rather large and intact).

3) The technique is extremely simple.

Disadvantages of the method:

1) The stain fades rapidly so that even after 60 seconds there is some discoloration (This, however, may at times be an advantage since the method does not damage the tissue or leave permanent changes so that other methods of study may be applied to the same specimen).

2) Occasionally it is not possible to differentiate between a small vessel and a fiber without a lumen.

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Fig. 1.

Fig. 2.

Fig. 3.

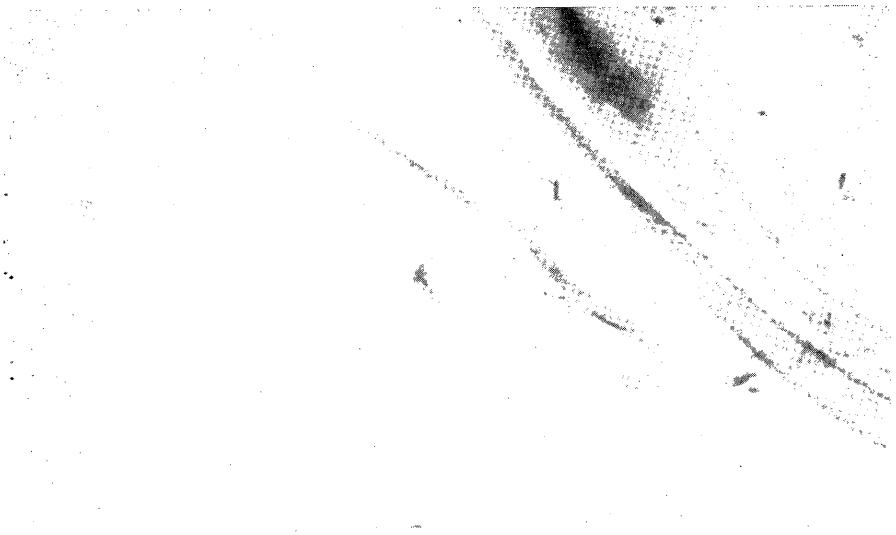

Fig. 4.

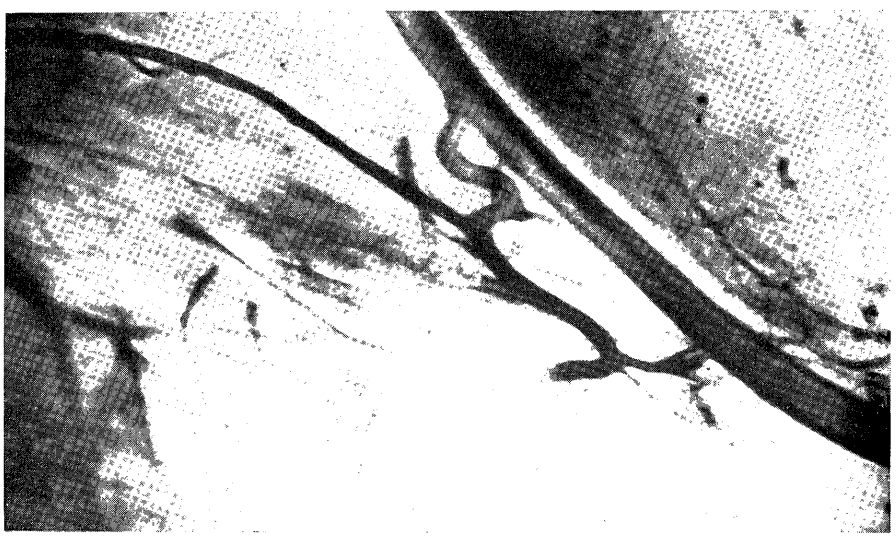

Fig. 5.

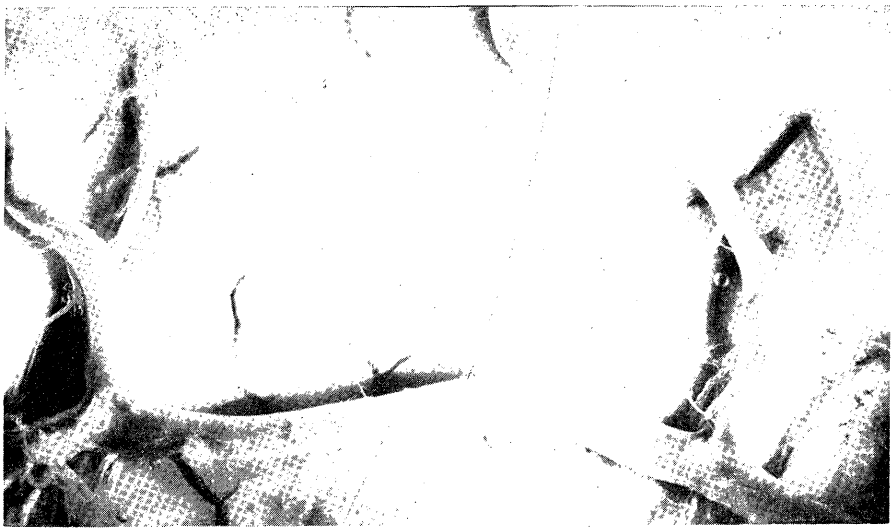

Fig. 6.

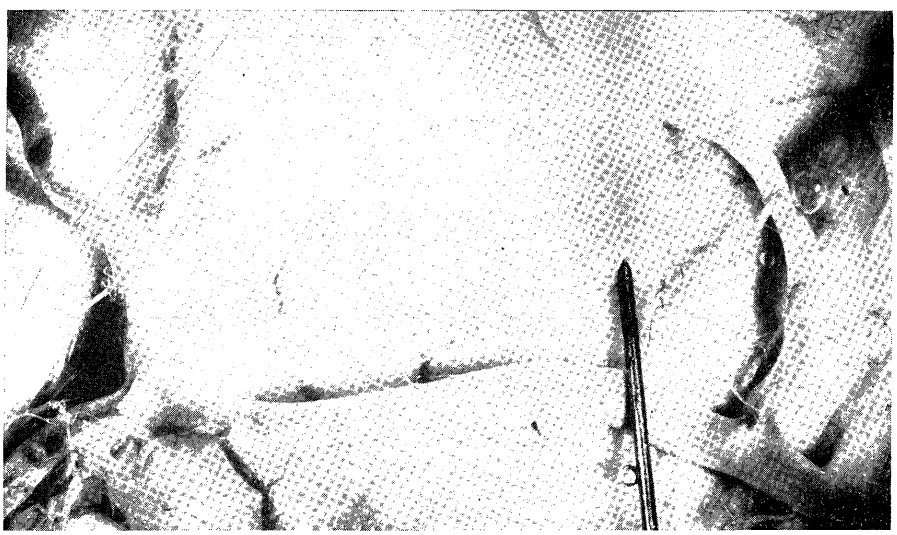

Fig. 7.

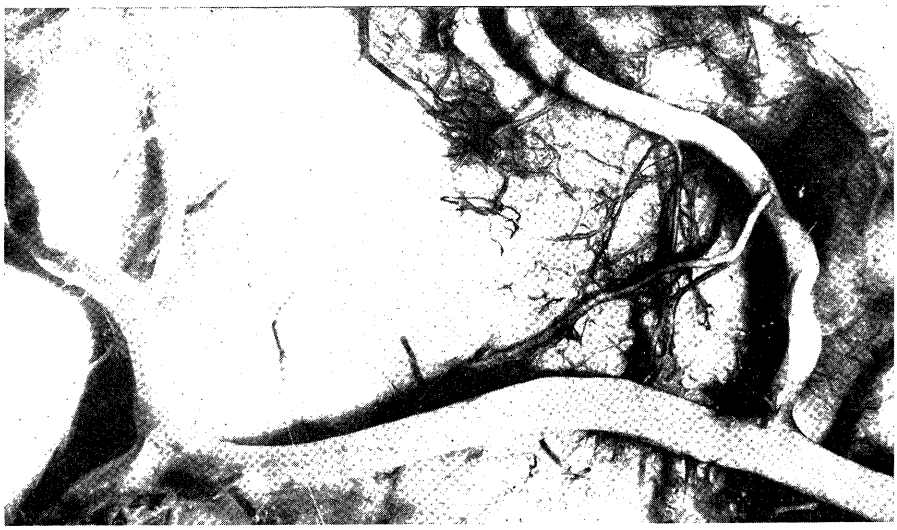

Fig. 8.

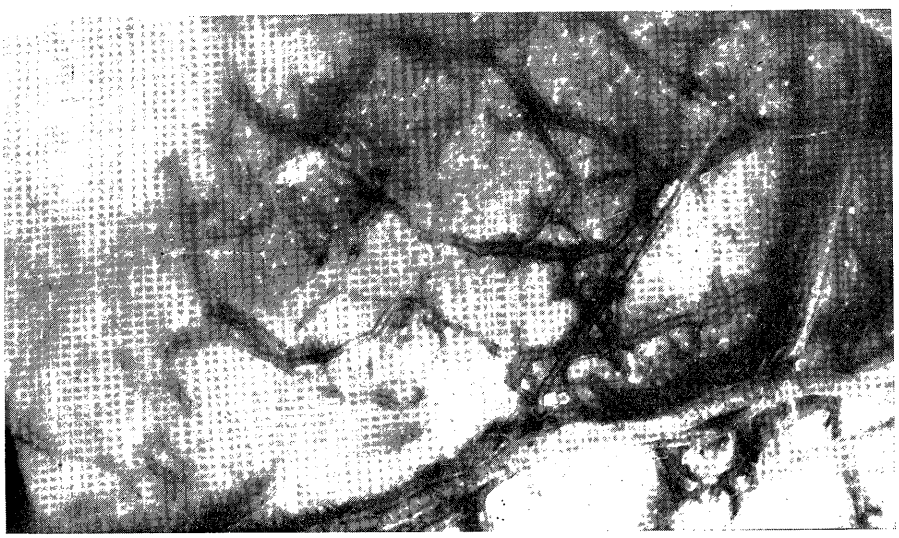

Fig. 9.

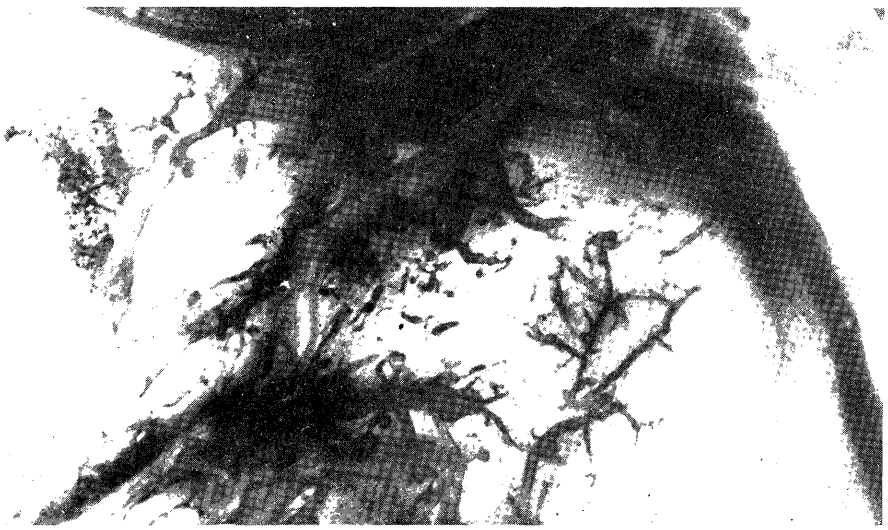

Fig. 10.

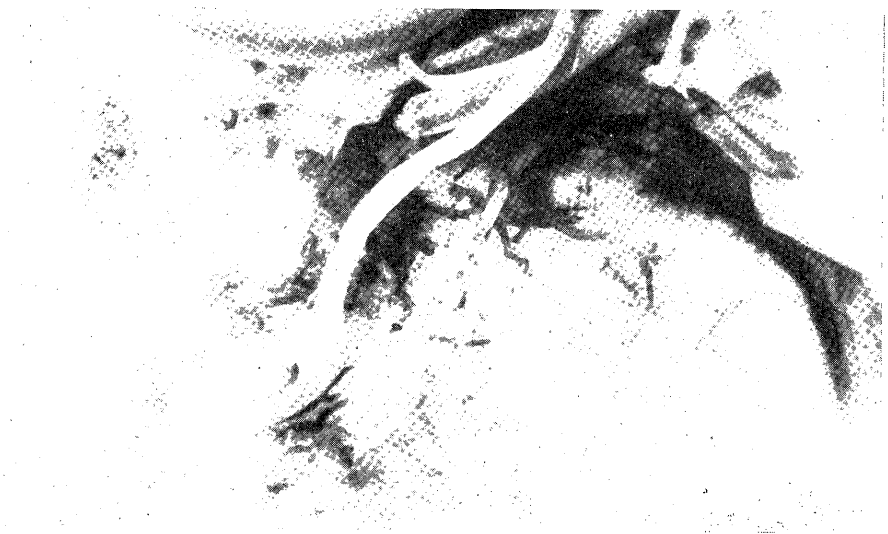

Fig 11.

Fig. 12.